Second-Trimester Amniotic Fluid or Maternal Serum Interleukin-10 Levels and Small for Gestational Age Neonates

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Objective: To evaluate if interleukin-10 levels in either early second-trimester amniotic fluid (AF) or maternal serum can be utilized as a predictor of the subsequent occurrence of small for gestational age (SGA) infants after controlling for gestational age at delivery.

Methods: We identified patients who underwent genetic amniocentesis for standard genetic indications or maternal blood sampling for maternal serum alpha-fetoprotein (MSAFP)/triple screen between January 1992 and February 1995 with available follow-up delivery data. Small for gestational age was defined as birth weight less than the tenth percentile for gestational age. Control patients were matched for gestational age at delivery, maternal age, race, and parity with at least two controls for each study patient. We excluded patients with maternal immune disease, chronic hypertension, diabetes, asthma, congenital heart disease, multiple gestation, and fetuses with structural or chromosomal anomalies. Second-trimester AF and serum samples were assayed for interleukin-10. Potential confounding variables considered were MSAFP level, smoking history, pregnancy-induced hypertension, and neonatal gender. The interleukin-10 levels were normalized using natural log transformation for statistical analysis. Statistical analysis included χ^2, Fisher exact test, and analysis of variance, with $P < .05$ considered significant.

Results: From the AF data base, 18 patients (6%) delivered SGA neonates and were matched with 46 controls. From the maternal serum data base, 13 patients (7%) delivered SGA neonates and were matched with 45 controls. Neither AF nor maternal serum interleukin-10 levels were significantly different in patients subsequently delivering SGA neonates compared with controls (AF: median 21.0 pg/mL [range 13.8–27.6] versus 17.5 pg/mL [range 8.9–362.12], $P = .18$; serum: median 15.7 pg/mL [range 9.9–73.5] versus 18.7 pg/mL [range 9.7–71.7], $P = .60$, respectively). No significant differences were identified in gestational age at sampling, maternal smoking history, pregnancy-induced hypertension, or elevated MSAFP in patients delivering SGA neonates compared with controls ($P > .05$ for each). As expected, birth weight was significantly lower in patients delivering SGA neonates compared with controls ($P < .001$).

Conclusion: Second-trimester AF or maternal serum interleukin-10 levels are not predictive of subsequent delivery of SGA infants. *(Obstet Gynecol 1996;88:24–8)*

Intrauterine growth restriction (IUGR) is associated with a high risk of fetal death and accounts for an important portion of perinatal mortality.[1] Nearly one-third of patients with IUGR have no risk factors.[2,3] Morphometry studies from the pathology literature,[4,5] validated by functional studies utilizing Doppler velocimetry[6] of the maternal and fetal circulation, suggest that idiopathic fetal growth restriction represents a heterogeneous disorder and, in most cases, the causal insult occurs at the time of placentation.[7,8] The detection of early predictors of patients at risk for IUGR has the potential to allow patients to modify behavior (increased maternal rest) and may open the venue for studies on the value of prophylactic treatment (ie, low-dose aspirin) to prevent the occurrence of small for gestational age (SGA) neonates.

Interleukin-10, a potent immunosuppressive cytokine, has several functions, including the inhibition of macrophage activity and function, in vivo suppression of cell-mediated immunity, and the inhibition of nitric oxide production.[9,10] The presence of interleukin-10 appears advantageous during autoimmunity and trans-

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plantation,[9,11] possibly explaining the presence in pregnancy. Interleukin-10 has been found in pregnant women and localized by immunohistologic methods to the interface area between maternal and fetal tissues and expressed at high levels in placental tissues.[9] Although the role of interleukin-10 in the physiology of human gestation has not been demonstrated, interleukin-10 may play a role in protecting the fetus from maternal immunorejection.

Elevated levels of interleukin-10 have been reported in second-trimester amniotic fluid (AF) of patients subsequently delivering SGA infants compared with controls who delivered appropriate for gestational age (AGA) infants at term.[12] However, the rate of SGA infants is higher among preterm than term deliveries.[13–15] Our study was designed to evaluate if interleukin-10 levels either in AF or maternal serum can be utilized as a predictor of the subsequent occurrence of SGA infants after controlling for gestational age at delivery.

Materials and Methods

In this case-control study, we utilized a data base of women who underwent second-trimester amniocentesis for standard genetic indications or maternal blood sampling for maternal serum alpha-fetoprotein (MSAFP)/triple screen between January 1992 and February 1995 and for whom pregnancy outcome information was available. Patients received genetic counseling and consented for inclusion in the study. We excluded patients with maternal immune disease, chronic hypertension, diabetes, asthma, congenital heart disease, multiple gestation, and fetuses with structural or chromosomal anomalies. Gestational age was confirmed or established by ultrasonographic fetal biometry at time of amniocentesis (less than 20 weeks' gestation). Small for gestational age was defined as birth weight less than the tenth percentile for gestational age, based on California birth weight curves.[1] These curves have a similar racial constituent and sea-level population. Control patients, delivering an AGA infant, were chosen from the same data base and matched for gestational age at delivery, maternal age, race, and parity, blinded to all other variables, with at least two controls for each study patient. Potential confounding variables considered were elevated MSAFP greater than two multiples of the median, smoking history, pregnancy-induced hypertension, and neonatal gender. Demographic data collected by chart review included gestational age at amniocentesis, maternal age, race, parity, MSAFP, gestational age at delivery, development of pregnancy-induced hypertension, neonatal gender, and birth weight.

The AF specimens were centrifuged (100–150 × *g* for 9 minutes) to remove cellular components for karyotype determination, and the supernatant samples were frozen at −20C. Maternal blood specimens were centrifuged (100–150 × *g* for 9 minutes) to remove cellular components, and the serum samples were frozen at −20C. Second-trimester AF and serum samples were assayed by an enzyme-linked immunoassay (ELISA) kit specific for interleukin-10 (Endogen, Cambridge, MA). The ELISA was validated for AF and the detection limit was 5.6 pg/mL. The intra-assay and inter-assay coefficient of variation for AF were 7.6 and 8.3%, respectively. For maternal serum, the ELISA detection limit was 3 pg/mL. The intra-assay and inter-assay coefficient of variation for maternal serum were both less than 10%. The ELISA is specific for human interleukin-10 and does not cross-react with any other cytokine. Interleukin-10 values were normalized using natural log transformation for statistical analysis.

Statistical analysis included Fisher exact test and analysis of variance, with $P < .05$ considered significant. Assuming this test will be used as a clinical diagnostic procedure when a single measurement is made on a patient and a decision determined, for 85% sensitivity and specificity, the two populations would need to differ in mean values by two standard deviations. A power analysis indicates that in an unpaired test, six samples in each group would give 80% power at $\alpha = .05$ when two populations differ by two standard deviations. Our sample size is adequate to detect a difference large enough for this test to be useful in a diagnostic setting.[16] The study was approved by both the Institutional Review Board Committees at Georgetown University and the National Institute of Child Health and Human Development.

Results

Interleukin-10 was detected in all samples of both AF and maternal serum. One control and three study subjects were in both the AF and maternal serum data bases.

From the AF data base, 18 patients (6%) delivered SGA neonates (SGA group) and were matched with 46 controls (mean gestational age at sampling 17.3 ± 2.9 weeks and 16.6 ± 2.9 weeks, respectively; $P = .4$). Maternal characteristics, epidemiologic risk factors for growth restriction, and pregnancy outcome were not significantly different between the patients with subsequent growth restriction and controls.

As expected, based on matching criteria, the SGA and control groups in the AF data base were not different for gestational age at delivery (37.2 ± 3.3 versus 36.9 ± 3.9 weeks; $P = .8$), maternal age (36.7 ± 4.4 versus 36.1 ±

3.1 years; $P = .5$), frequency of African-American race (four [22%] versus eight [17%]; $P = .2$), and nulliparity (13 [72%] versus 38 [83%]; $P = .2$). Known epidemiologic risk factors for SGA were not different between the SGA and control groups, including rate of elevated MSAFP, defined as greater than two multiples of the median (one [5%] versus one [2%]; $P = .4$), maternal tobacco use (one [5%] versus one [2%], $P = .4$), pregnancy-induced hypertension (zero in both), and neonatal female gender (11 [61%] versus 27 [59%]; $P = .2$), respectively. As expected, birth weight was significantly lower in SGA patients (2203 ± 662 versus 3053 ± 881 g; $P < .001$).

The interleukin-10 levels were normalized using natural log transformation for statistical analysis. Interleukin-10 values were not significantly different in AF (median 21.0 pg/mL [range 12.8–27.6] versus 17.5 pg/mL [range 8.9–362.1]; $P = .18$) between the SGA group and controls. Logistic regression analysis showed no significant dependence of the occurrence of SGA on interleukin-10 values (odds ratio 0.6–7.1). Figure 1 illustrates the interleukin-10 values in the SGA and control groups. Figure 2 demonstrates the distribution of the AF interleukin-10 levels according to the gestational age at delivery in the two groups.

From the maternal serum data base, 13 patients delivered SGA neonates (7%) and were matched with 45 controls (mean gestational age at sampling 16.2 ± 1.1 and 16.8 ± 1.5 weeks, respectively; $P = .4$). As expected, from the matching criteria, the SGA and control groups were not significantly different for gestational age at delivery (36.9 ± 4.7 versus 37.9 ± 3.0 weeks; $P = .4$), maternal age (28.5 ± 9.4 versus 31.9 ± 4.1 years; $P = .07$), frequency of African-American race (six [46%] versus 11 [24%]; $P = .2$), and nulliparity (five [38%] versus 21 [47%]; $P = .2$). Known risk factors for SGA were also not different between the SGA and control groups, including rate of elevated MSAFP (one [8%] versus one [2%]; $P = .4$), maternal tobacco use (zero versus two [4%]; $P = .6$), pregnancy-induced hypertension (zero in both), and neonatal female gender (ten [76%] versus 25 [56%]; $P = .3$), respectively. As expected, birth weight was significantly lower in subjects with SGA neonates (2138 ± 660 versus 3238 ± 747 g; $P < .001$).

Maternal serum interleukin-10 levels were not significantly different (median 15.7 pg/mL [range 9.9–73.5] versus 18.7 pg/mL [range 9.7–71.7]; $P = .60$) in women subsequently delivering SGA neonates compared with controls, respectively. The maternal serum interleukin-10 levels in patients who delivered SGA neonates and controls is illustrated in Figure 3. Maternal serum interleukin-10 levels in relation to gestational age at delivery are shown in Figure 4.

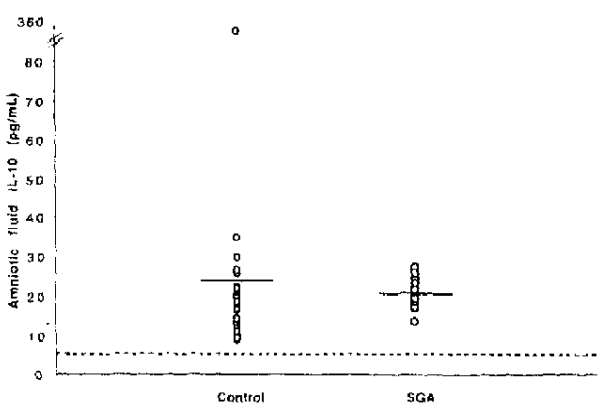

Figure 1. Distribution of amniotic fluid interleukin (IL)-10 values in pg/mL for control patients and patients delivering small for gestational age (SGA) neonates. The sensitivity of the enzyme-linked immunosorbent assay (5.6 pg/mL) is represented by a dotted line; the mean in each group is represented by a bar.

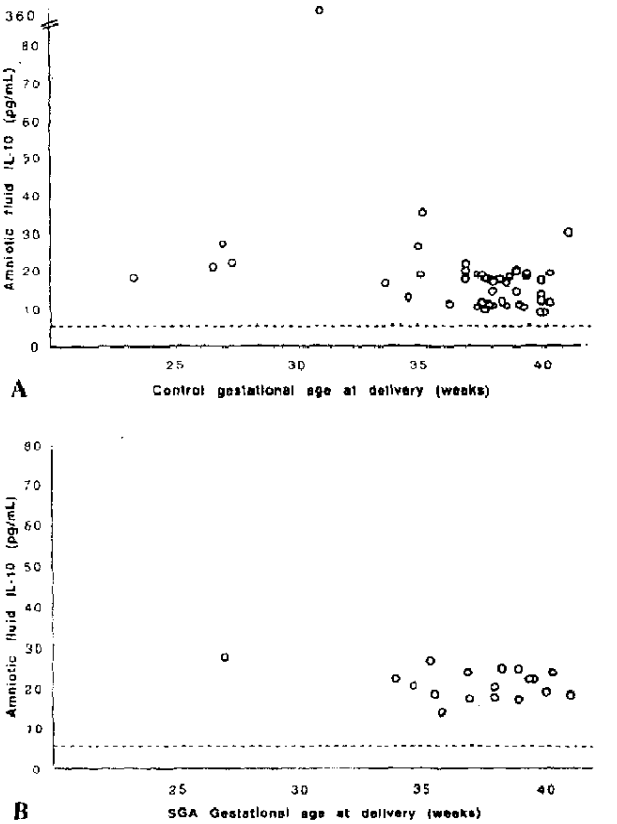

Figure 2. Amniotic fluid interleukin (IL)-10 values in pg/mL versus gestational age at delivery in (A) control patients and (B) patients delivering small for gestational age (SGA) neonates. The sensitivity of the enzyme-linked immunosorbent assay (5.6 pg/mL) is represented by a dotted line.

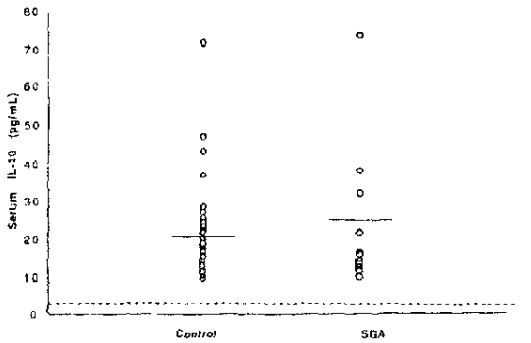

Figure 3. Distribution of maternal serum interleukin (IL)-10 values in pg/mL for control patients and patients delivering small for gestational age (SGA) neonates. The sensitivity of the enzyme-linked immunosorbent assay (≤3 pg/mL) is represented by a dotted line; the mean in each group is represented by a bar.

Discussion

We found no significant difference in early second-trimester AF interleukin-10 levels between patients subsequently delivering SGA infants and AGA controls, after controlling for gestational age at delivery. In addition, we found no association between maternal serum early second-trimester values of interleukin-10 and the delivery of SGA neonates. Our results are at variance with a previous study by Heyborne et al,[12] which reported an association between elevated second-trimester AF interleukin-10 values and subsequent delivery of SGA neonates compared with AGA controls who delivered at term. The source and stimulus for interleukin-10 in association with IUGR remains unclear.

Different reasons may explain the variances between the two studies. The study by Heyborne et al[12] did not control for gestational age at delivery. Their control group included term AGA subjects with significantly prolonged gestations compared with the SGA subjects ($P < .02$). Because the prevalence of SGA infants is higher among preterm compared with term deliveries,[13–15] the observed difference in interleukin-10 levels between SGA and AGA infants may be accounted for by the difference in gestational age at delivery. Because our case-control study matched for risk factors of growth restriction, we are unable to evaluate if interleukin-10 is a predictor of preterm delivery. In addition, in the study of Heyborne et al,[12] a significant difference in the rate of nulliparity was present between the SGA and control groups, with a significant association between elevated interleukin-10 levels and nulliparity ($P = .003$) in the SGA group.

Finally, in the published study,[12] the sensitivity of the ELISA was 40 pg/mL, and the median in the AGA term control group was below the detectable range. An abnormal test, defined as greater than 40 pg/mL (the test's limit of sensitivity), predicted SGA gestations with a sensitivity of 63%. Although the assay used for interleukin-10 was different in our study, their assay was unable to detect interleukin-10 in many samples. The Endogen assay, with a sensitivity of 5.6 pg/mL in AF, was able to detect interleukin-10 in all our AF samples, with values similar to Greig et al.[10] Defining an abnormal test as any level that is detectable in the assay may have compromised the demonstrated significance. If interleukin-10 is a mediator of IUGR, perhaps it is not activated to a detectable degree in the second-trimester, either locally in the AF or systemically in the maternal serum. Alternatively, because fetal growth restriction is associated with different etiopathogenic mechanisms, interleukin-10 may be a marker of only one of the etiologic factors involved.

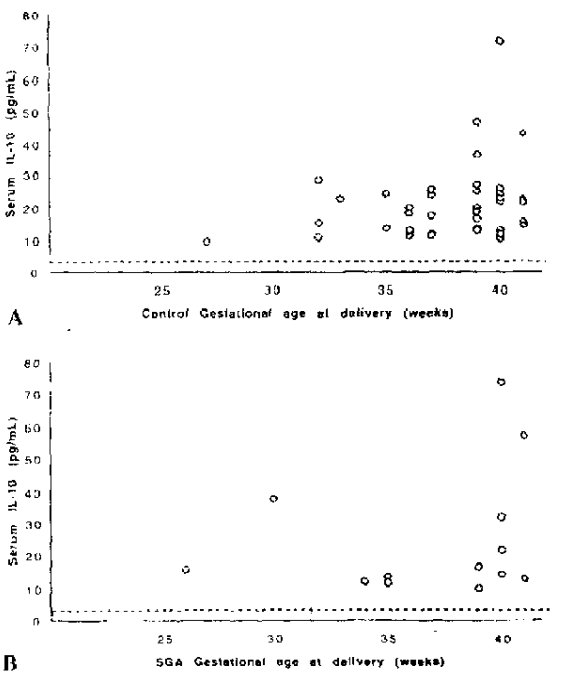

Figure 4. Maternal serum interleukin (IL)-10 values in pg/mL versus gestational age at delivery in (A) control patients and patients delivering (B) small for gestational age (SGA) neonates. The sensitivity of the enzyme-linked immunosorbent assay (≤3 pg/mL) is represented by a dotted line.

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